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# PRE-APPEAL BRIEF REQUEST FOR REVIEW

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I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to "Mail Stop AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450" [37 CFR 1.8(a)]

on August 10, 2007

Signature [signature]

Typed or printed name Renato Marco Domingo

| Application Number | Filed |
|---|---|
| 10/750,005 | December 30, 2003 |

First Named Inventor: Herbert T. Nagasawa

| Art Unit | Examiner |
|---|---|
| 1654 | Thomas Heard, Ph.D. |

Applicant requests review of the final rejection in the above-identified application. No amendments are being filed with this request.

This request is being filed with a notice of appeal.

The review is requested for the reason(s) stated on the attached sheet(s).
Note: No more than five (5) pages may be provided.

I am the

- ☐ applicant/inventor.
- ☐ assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)
- ☑ attorney or agent of record. Registration number 34,470
- ☐ attorney or agent acting under 37 CFR 1.34. Registration number if acting under 37 CFR 1.34 _____

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NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☑ *Total of 1 forms are submitted.

# PRE-APPEAL BRIEF REQUEST FOR REVIEW ATTACHMENT

## A. *The Claimed Invention*

Claims 1-4, 9-10, 20-21, 25-26, 46 and 50-51 are pending. The independent claims of the invention are as follows.

1. A method for reducing oxidative stress in a cell of a subject comprising contacting the cell with a ***sulfhydryl protected glutathione prodrug*** so as to reduce oxidative stress in a cell, wherein the sulfhydryl protected glutathione prodrug is L-CySSG, GSSMA, GSSME, S-Ac-GSH-OEt, a derivative thereof or a pharmaceutically acceptable salt thereof.

20. A method for increasing glutathione levels in a cell comprising administering to a subject a ***sulfhydryl protected glutathione prodrug*** so as to increase glutathione levels in a cell, wherein the sulfhydryl protected glutathione prodrug is L-CySSG, GSSMA, GSSME, S-Ac-GSH-OEt, a derivative thereof or a pharmaceutically acceptable salt thereof.

46. A method for reducing hepatotoxicity comprising administering to a subject a ***sulfhydryl protected glutathione prodrug*** so as to reduce hepatotoxicity, wherein the sulfhydryl protected glutathione prodrug is L-CySSG, GSSMA, GSSME, S-Ac-GSH-OEt, a derivative thereof or a pharmaceutically acceptable salt thereof.

Before Applicants' invention, no one taught or suggested using sulfhydryl protected glutathione prodrugs to replenish GSH in a subject. Applicants were the first to use sulfhydryl protected glutathione prodrugs as a readily accessible source of GSH. Applicants were the first to provide experimental evidence that an exogenously administered sulfhydryl protected glutathione prodrug (e.g., L-CySSG) can protect the liver from the toxic insult of acetaminophen, a drug known to severely deplete GSH and elicit hepatotoxicity.

**Advantage of the invention:** A sulfhydryl protected glutathione prodrug releases a preformed glutathione making it immediately available to the cell to reduce oxidative stress, increase GSH and/or reduce hepatotoxicity. Release of GSH from a sulfhydryl protected glutathione prodrug bypasses the cellular GSH synthesis pathway providing GSH independent of whether the pathway is functional or not. Additionally, the sulfhydryl protected glutathione prodrug protects GSH from degradation by the cell.

For example, in an embodiment of the invention, the sulfhydryl protected glutathione prodrug is CySSG (also known as CSSG), where CySSG is a mixed disulfide of L-cysteine and glutathione. When reduced, CySSG releases a preformed glutathione as well as L-cysteine. The L-cysteine is a precursor for *de novo* glutathione synthesis. Thus, CySSG produces **two** glutathiones; the first glutathione is released from CySSG by reduction making it immediately available to the cell; the second glutathione is *de novo* synthesized by the cell from the L-cysteine that was released by CySSG (specification at page 9, first paragraph).

### *B. Rejection under 35 U.S.C. §103(a)*

The Office rejected the pending claims as unpatentable over Shirota et al., Jonas et al. and Bender et al. **The Office took the following positions.**

1. The combined prior art references suggested the present invention because:
    a. it would have been obvious to substitute CySSG (Jonas et al.) for CySSME (Shirota et al.) for the production of GSH through cysteine production (April 11, 2007, Office Action at page 4, paragraph 2 and page 11, last paragraph); and
    b. one skilled in the art would recognize the nexus between cysteine production and GSH levels, important for regulating oxidative stress (April 11, 2007, Office Action at page 3, paragraph 3; page 6, last paragraph to page 7, line 2; and page 11, paragraph 2).
2. One skilled in the art would have been motivated to administer CySSG to induce intracellular Cystine (as described in Jonas et al.), whereupon the cell converts cystine to cysteine, the rate limiting precursor of GSH, and the reduction of oxidative stress (Bender et al.; April 11, 2007 Office Action at page 3, paragraph 3).
3. One of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention (i.e., a method for reducing oxidative stress using a sulfhydryl protected glutathione prodrug) because CSSG has already been administered to cells and cysteine levels were increased three fold (April 11, 2007 Office Action at page 3, paragraph 3 and page 10, paragraph 2).
4. CySSG is NOT a sulfhydryl protected glutathione prodrug (as asserted by Applicants) but rather a cysteine prodrug (April 11, 2007, Office Action at page 4, last line to page 5, line 3; page 8, paragraph 2; and page 10, paragraph 2).

### *C. Argument*

***Teachings of the Cited Art***

Shirota et al. teach that L-cysteine prodrugs such as CySSME (a mixed disulfide of cysteine and mercaptoethanol), can reduce acetaminophen induced hepatic toxicity due to enhanced GSH synthesis and maintenance of hepatic GSH homeostasis. CySSME is not a sulfhydryl protected glutathione prodrug. Shirota does not teach the use of a sulfhydryl protected glutathione prodrug.

Jonas et al. teach a use of a sulfhydryl protected glutathione prodrug, i.e. CSSG, to provide "a soluble source of cyst(e)ine" for cystinotic cells, cells from patients heterozygous for cystinosis and normal (i.e., non-cystinotic) cells to increase cystine levels in the cells. Jonas did not teach or suggest use of CSSG to increase GSH levels in a cell.

Bender teaches that in cultured astrocytes cellular uptake of cystine is the rate-limiting step in GSH biosynthesis. Cystine, after transport into cells, is reduced to cysteine, a precursor of GSH. Thus, cystine, via cysteine, is required for maintaining cellular levels of GSH. GSH protects cells

against oxidative stress and various toxins. Bender does not teach or suggest increasing GSH levels using cystine or any other molecule, let alone a sulfhydryl protected glutathione prodrug.

***The Prior Art References Did Not Suggest the Invention***

The Office alleges that the combination of cited references suggested the claimed invention since Bender's teaching (cystine is reduced to cysteine which is used to *de novo* synthesize glutathione) provides a nexus for substituting CSSG (Jonas et al.) in place of a L-cysteine prodrug such as CySSME (Shirota et al.) for the production of GSH thereby reducing oxidative stress (April 11, 2007 Office Action at page 3, paragraph 3; page 6, last paragraph to page 7, line 2; and page 11, paragraph 2).

This reasoning falsely assumes that:
(1) there is similarity between CSSG and CySSME which would suggest their interchangeability;
(2) there is motivation to use sulfhydryl protected glutathione prodrug instead of a cysteine prodrug; and
(3) motivation exists to use a sulfhydryl protected glutathione prodrug to reduce oxidative stress.

There is no similarity between CSSG and CySSME. CSSG is a sulfhydryl protected *glutathione* prodrug. In contrast, CySSME is a sulfhydryl protected *cysteine* prodrug (specification at page 3, lines 9-12). CySSME is a mixed disulfide of L-cysteine and mercaptoethanol. Only a single glutathione can be produced from CySSME, this via *de novo* synthesis.

There is no motivation to substitute CSSG for CySSME for reducing oxidative stress in a cell. CSSG and CySSME do not share a common utility and function (In re Lalu and Foulletier, 747 F.2d 703, 223 USPQ 1257 (Fed. Cir. 1984)). They are not equivalents.

As the Office states, Shirota teaches that hepatoprotection by L-cysteine generated from a prodrug is due to enhanced GSH **synthesis** (April 11, 2007, Office Action at page 10, paragraph 3). Neither Jonas nor Bender teach or suggest what Shirota fails to teach or suggest, namely, the use of sulfhydryl protected glutathione prodrug to deliver **preformed** glutathione to cells or to reduce oxidative stress in a cell. Accordingly, the combination of the primary and secondary references does not and cannot render obvious the claimed methods.

The mere fact that references can be combined does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. In re Mills, 916 F.2d 680, 16 U.S.P.Q.2d 1430 (Fed. Cir. 1990), cited in MPEP §2143.01. There must be a reason or suggestion in the art for modifying the prior art other than the knowledge learned from Applicants' disclosure[1]. However, the cited references provide none.

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[1] In re Dow Chemical Co., 837 F.2d 469, 473, 5 USPQ2d 1529, 1532 (Fed. Cir. 1988).

*The Benefits of Using a Sulfhydryl Protected Glutathione Prodrug*

Unlike sulfhydryl protected *cysteine* prodrugs, administration of a sulfhydryl protected *glutathione* prodrug to a cell provides a source of *preformed glutathione* independent of the cell's endogenous GSH biosynthesis pathway. As an organism ages, the GSH synthesis pathway becomes impaired leading to a decrease in GSH biosynthesis (Hazelton, G.A. and Lang, C.A., 1980, Glutathione Contents of Tissues in the Aging Mouse. *Biochem. J.* 188, 25-30). Sulfhydryl protected glutathione prodrugs such as CSSG deliver preformed GSH to the cell whether or not the GSH biosynthesis pathway is functional.

Additionally, to alleviate aggressive oxidative stress, for example after acetaminophen administration, a source of GSH such as a sulfhydryl protected glutathione prodrug can be provided expeditiously to a cell. Prior to the present invention, it was not obvious that sulfhydryl protected glutathione prodrugs were a readily available and efficient source of GSH to a cell nor to administer a sulfhydryl protected glutathione prodrug such as CSSG since, among other things, it had not been known whether the enzymatic reduction (or enzyme-catalyzed thiol-disulfide interchange reaction) velocity of CSSG would be sufficiently fast and efficient to replenish GSH.

*There Was No Reasonable Expectation of Success for the Substitution of a Sulfhydryl Protected Glutathione Prodrug for a Cysteine Prodrug*[2]

In response to the Applicants statement that there was no reasonable expectation of success that the substitution of a sulfhydryl protected glutathione prodrug for a cysteine prodrug, sufhydryl-protected or otherwise, would produce the claimed methods, the Office stated that "[b]oth CySSME and CYSSG (CSSH) (sic) were shown to produce an increase in cysteine levels which are important for producing GSH. Therefore, it worked and was successful" (April 11, 2007, Office Action at page 10, paragraph 2).

Respectfully, the Office misses the point. Although GSH can be generated from cysteine prodrugs by *de novo* synthesis in a cell, whether cysteine is converted into GSH depends on several factors: the type of cell (Hazelton and Lang, *supra*), the age of the cell (Hazelton and Lang, *supra*), the amount of cysteine present (Richman, P. and Meister, A., 1975, Regulation of Gamma-Glutamyl-L-Cysteine Synthetase by Nonallosteric Feedback Inhibition by Glutathione, *J. Biol. Chem.* 250, 1422-1426), the amount of GSH present (Richman and Meister, *supra*), etc. For example, if a cell has sufficient GSH, the GSH synthesis pathway may be turned off. Alternatively, the GSH pathway may be blocked by an inhibitor (Griffith, O.W. and Meister, A., 1979, Potent and Specific Inhibition of Glutathione Synthesis by Buthionine Sulfoximine (S-n-butyl homocysteine sulfoximine), *J. Biol. Chem.* 254, 7558-7560) or impaired (Larsson, A. and

[2] In the Advisory Action dated August 6, 2007, Applicants respectfully point out that the Office's summary of Applicants' position is inaccurate and the actual arguments are found in this section.

Hagenfeldt, L., 1983, Hereditary Glutathione Synthetase Deficiency in Man. *Functions of Glutathione: Biochemical, Physiological, Toxicological, and Clinical Aspects*, Raven Press, New York). Whether a molecule can increase cysteine is not pertinent to the claimed invention.

Moreover, not all compounds containing cysteine (e.g., cysteine prodrugs) are substitutable for CySSME to increase cysteine levels. Some cysteine prodrugs may increase cysteine levels but others do not (Crankshaw et al., *J Biochem Mol Tox*, 2002, 16(5):235-244).

Even if, *arguendo*, CSSG could be substituted for CySSME to increase cysteine levels, the claims are not directed to increasing cysteine levels. *The claims are directed to uses of sulfhydryl protected glutathione prodrugs to reduce oxidative stress, increase GSH in a cell or reduce hepatotoxicity.* In the claimed methods, the GSH can be rapidly released from the prodrug and provided to the cell even if the endogenous cellular GSH biosynthesis pathway is broken. The sulfhydryl protected glutathione prodrug protects GSH from degradation by the cell.

Jonas administered CSSG to increase intracellular cysteine, but did not disclose that CSSG administration would provide preformed GSH to reduce oxidative stress in a subject as claimed. The Shirota and Bender references do not supply what Jonas lacks. Given the state of the art, there would have been no reasonable expectation that one would be able to produce the claimed method by simply administering to a subject a sulfhydryl protected glutathione prodrug such as CSSG.

Although obviousness under 35 U.S.C. §103 does not require absolute predictability of success, 35 U.S.C. §103 does require a reasonable expectation of success to find obviousness. (In re O'Farrell, 853 F.2d 894, 7 USPQ2d 1673 (Fed. Cir. 1988)).

***CySSG Is a Sulfhydrydral Protected Glutathione Prodrug***

The Office's statement that CySSG is NOT a sulfhydryl protected glutathione prodrug is contrary to the definition of the generally accepted term "prodrug" (a dictionary definition was attached to the response to the April 11, 2007, Office Action) as well as contrary to the specification at page 3, lines 9-14. MPEP §2111.01 provides that the Office must give the words of the claim their plain meaning. This is especially important when applicants provide a definition of a claim term in accord with its accepted meaning.

***Conclusion***

Given the stated arguments above, Applicants respectfully request allowance of the pending claims.